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Postinfection Control in T4 Bacteriophage Infection: Inhibition of the *rep* Function

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We suggest that the general mechanism by which T4 phage turns off host macromolecular synthesis involves specific phage proteins which react with key components in the synthetic pathway. Support for this mechanism exists for the inhibition of host RNA synthesis. Here we note that the host *rep* function was inhibited after T4 phage infection. Since *rep* functions are known to be involved in host DNA replication, inhibition of *rep* might alter the course of host DNA replication.

It has been known for some time that the synthesis of host-specific macromolecules is terminated after T4 phage infection (see review by Koerner and Snustad [15]). Initially it was thought that these synthetic activities were terminated because of the destruction of the host nuclear apparatus. Subsequent experiments have shown that the host DNA has few nicks and appears to be mainly intact in neutral sucrose gradients at a time when host macromolecular synthesis is terminated (30). Despite evidence for some degradation, the mechanism of shutoff may be independent of degradation. We favor the view that the course of host macromolecular synthesis is altered because of positive control functions introduced by the phage. An example of such a control is the inhibition of the *Escherichia coli recBC* nuclease activity (28, 34) by a protein inhibitor produced by the phage (3). Another example is the ADP ribosylation of *E. coli* RNA polymerase by T4 phage-induced enzymes (9). This modification has been proposed as the basis for the shift in transcription from host to phage template (20). However, the significance of ADP ribosylation has been questioned (14), and Koerner and Snustad (15) have suggested that one of the T4 phage-induced polypeptides which is complexed with RNA polymerase (31, 32) might be responsible for the shutoff of host RNA synthesis. In either case, the mechanism of the shutoff would involve interaction of phage-induced proteins with the synthetic enzyme.

In this communication, we report the inhibition of the host *rep* function by T4 phage. This observation is discussed in the context of a possible mechanism of control of host DNA replication.

MATERIALS AND METHODS

Bacterial and phage strains. The bacterial and phage strains used are indicated in Tables 1 and 2. *E. coli* strain MV12 was used to transfer the rep^+-containing plasmid pLC44-7 to strain HF4704 rep^- to make HF4704 rep^-(pLC44-7). HF4704 rep^-(pLC44-7) was isolated as lac^+ and was ϕX174 phage sensitive. When this strain was used for enzyme analysis, it was checked for dissociation of the plasmid by testing isolated colonies for sensitivity to ϕX174 and was found to be stable (40 of 40 isolates were rep^+).

Preparation of extracts. Extracts were prepared from *E. coli* cells which were grown at 37°C in M9 medium (21) (supplemented with 0.05% Casamino Acids; thy^- strains were further supplemented with 7 μg of thymine per ml). They were washed in buffer A (0.04 M Tris [pH 7.5]-0.01 M Na_3 EDTA-0.45 M sucrose), centrifuged, and frozen at −75°C. These preparations were thawed, suspended in buffer B (5 M NaCl-0.05 M $MgCl_2$-0.02 M Tris-0.002 M glutathione), disrupted in a French Pressure Cell, and centrifuged at 24,000 × *g* for 1 h. The supernatants were fractionated by the dextran-polyethylene glycol two-phase system (1), and the upper phase was dialyzed against buffer C (0.02 M Tris [pH 7.5]-0.01 M mercaptoethanol-0.001 M Na_3 EDTA-10% glycerol). All operations involving the preparation of the extracts and chromatography were carried out at 0 to 4°C.

Phosphocellulose chromatography. Extracts prepared from 3×10^{11} or 6×10^{11} cells were chromatographed on a P-11 phosphocellulose (Whatman Ltd.) column (0.9 by 4.5 cm) using 250 ml of buffer C with a linear KCl gradient. The gradient was monitored by conductivity measurements.

Assay of enzyme activity. DNA-dependent ATPase activity was assayed with [^{14}C]ATP or [^{3}H]ATP (New England Nuclear Corp.) and with native or denatured T4 DNA under conditions described previously (6, 25).

RESULTS

DNA-dependent ATPase activities and T4 phage infection. Phosphocellulose chromatography of extracts from T4 phage-infected and uninfected *E. coli* strain B indicated two major changes in the profiles of DNA-dependent ATPases. Extracts from cells infected with T4 dda^- phage for 10 min at 37°C had an additional

component, peak 5, which was absent in extracts from uninfected cells (Fig. 1A and B). We will identify this component in a later section. The other change was the disappearance of peak 3 in the extracts from T4 phage-infected cells. Previously it had been noted that the *recBC* nuclease, which is also a DNA-dependent ATPase, was inhibited after T4 phage infection (28, 34), but it seemed unlikely that peak 3 was the *recBC* nuclease because the *recBC* nuclease has little affinity for phosphocellulose (10).

Identification of the DNA-dependent ATPase inhibited by T4 phage. Based on preliminary evidence on the properties of the peak 3 enzyme, i.e., its activity as a DNA-dependent GTPase and its affinity for phosphocellulose, we guessed that the enzyme might be a *dnaB* or *rep* gene product. Extracts prepared from strain HF4704 *rep*$^-$ indicated that peak 3 was missing, whereas the wild-type strain HF4704 was active in peak 3 (Fig. 2A and B). Similar results were obtained with strains DLK12 and DLK13 (*rep*$^-$). These experiments were repeated and the results were confirmed with both sets of *rep*$^-$ and *rep*$^+$ strains. Although these experiments were performed with different strains, the profiles of DNA-dependent ATPase activities were very similar for *E. coli* strains B and HF4704 (Fig. 1 and 2), as well as for strains DLK12 and CR34 (data not shown). Also, we noted that the loss of the *rep* ATPase occurred in infections with all of several T4 mutants tested, including T4*amE*1140, T4*amN*82, T4*amNG*163 × 3, T4*amHL*628, and T4*uvsX*. The summation of the evidence indicates that the host *rep* enzyme disappears after T4 phage infection.

Because of the necessity of being certain of the identification of the *rep* protein, we introduced the ColE1-*rep*$^+$ plasmid into strain HF4704 *rep*$^-$. If peak 3 is the *rep* protein, the addition of the plasmid to strain HF4704 *rep*$^-$ should restore the activity in peak 3. Two separate isolates of HF4704 *rep*$^-$(pLC44-7) were grown under standard conditions and tested for DNA-dependent ATPase activity. Enzyme activities from one of these isolates is shown in Fig. 3. Note the restoration of activity in the peak 3 region (compare Fig. 2A and B) and the very high activity relative to the other peaks. This is consistent with the assumption that there are several plasmids per cell. Similar results were obtained from both isolates. These data further support the identification of the *rep* enzyme.

Effect of chloramphenicol. The loss of the *rep* DNA-dependent ATPase activity after T4 phage infection could be due to a natural instability of the enzyme, combined with the inhibition of host protein synthesis. Alternatively, it could result from the synthesis of a phage-induced inhibitor of *rep*. To test these alternatives, we added chloramphenicol (100 μg/ml) either together with the phage or 1 min before infection, and the incubation was continued for 10 min. In both cases, the *rep* activity disappeared from the peak 3 region (Fig. 4A). Coincidentally, there was the appearance of an activity in the area of fractions 38 to 40, suggesting that the *rep* enzyme was being altered. No similar activity occurs in the area of fractions 38 to 40 in normally infected cells, possibly because the enzyme is modified further. In the control, where chloroamphenicol alone was added, the *rep* enzyme was stable (Fig. 4B). These results force the conclusion that the loss of the *rep* activity is the result of phage infection. The observation that

TABLE 1. *Bacterial strains*

E. coli strain	Properties	Source
B	Wild type	Laboratory strain
HF4704 *thy*$^-$	*rep*$^+$	D. T. Denhardt
HF4704 *thy*$^-$ *rep*$^-$	*rep*$^-$	D. T. Denhardt
DLK12 (=MX223)	*rep*$^+$	D. T. Denhardt
DLK13 (=MX233)	*rep*$^-$	D. T. Denhardt
MV12	*lacY ΔtrpE5 thr leu recA* F$^+$/ pLC44-7	A. Kornberg (29)

TABLE 2. *Phage strains*

Phage	Properties	Source
T4*del*(39-56)10	Deletion mutant, DNA dependent, ATPase$^-$ (*dda*) (2)	T. Homyk and J. Weil (12)
T4*del*(39-56)1	*dda*$^+$	T. Homyk and J. Weil (12)
T4*amE*1140	Gene 62 mutant	R. S. Edgar
T4*amN*82	Gene 44 mutant	R. S. Edgar
T4*amHL*628	Gene 59 mutant	R. S. Edgar
T4*amNG*163 × 3	Gene 47 mutant	J. S. Wiberg
T4*uvsX*	UV sensitive	W. Harm
φX174	No plaques on *rep*$^-$ *E. coli* (7)	Laboratory strain

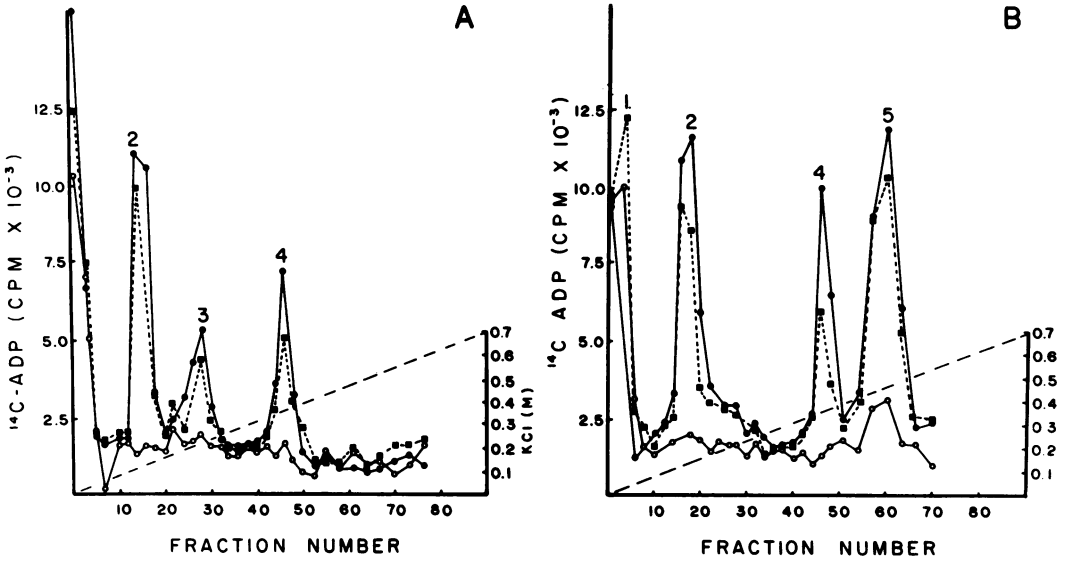

FIG. 1. *DNA-dependent ATPase activities after phosphocellulose column chromatography of extracts from T4-phage-infected and uninfected E. coli strain B. (A) Extracts from 3 × 10^{11} uninfected cells were chromatographed, 50-drop (approximately 2.75 ml) fractions were collected, and 0.03 ml was assayed for DNA-dependent ATPase activity with 0.015 mM [^{14}C]ATP (0.03 μCi; total volume, 0.15 ml). The assays were performed with native T4 DNA (■), denatured T4 DNA (●), and no DNA (○). (B) The experimental conditions were identical to those in A except that the extract was prepared from T4 phage-infected cells. The T4 phage strain used was T4 del(39-56)10, which is dda$^-$ (2).*

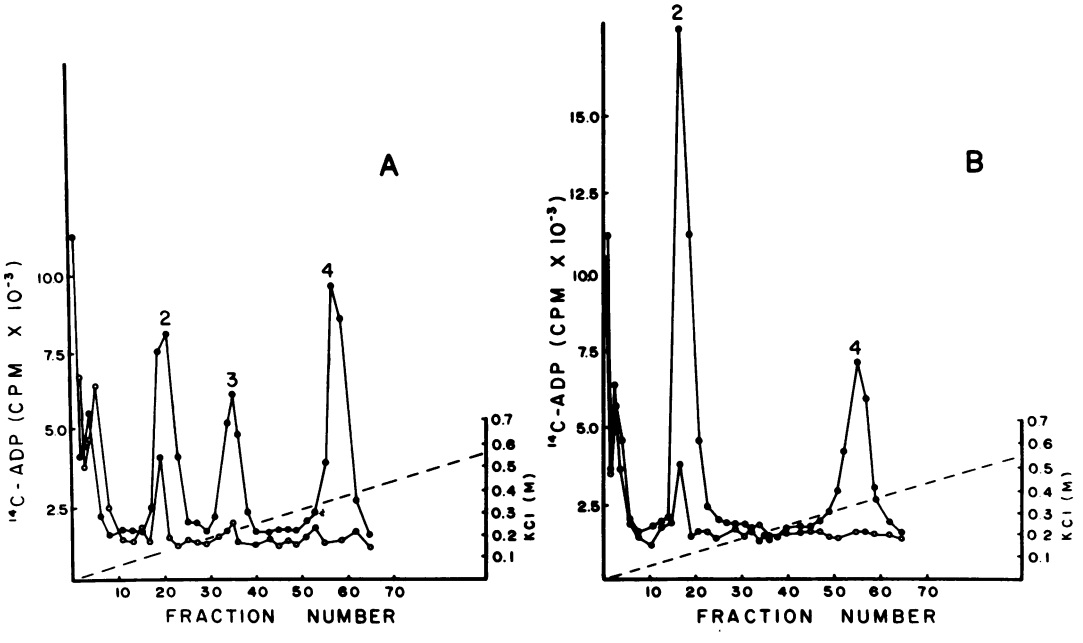

FIG. 2. *Phosphocellulose chromatography of extracts from rep$^+$ and rep$^-$ E. coli strains. (A) Analysis of DNA-dependent ATPase activities from the E. coli HF4704 rep$^+$ strain. The assays were performed with denatured T4 DNA (●) and no DNA (○). (B) Corresponding analysis of the mutant HF4704 rep$^-$. Extracts were prepared from 6 × 10^{11} cells.*

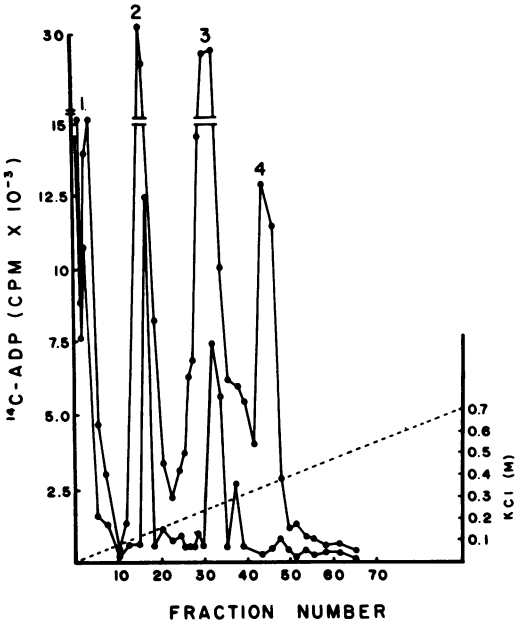

FIG. 3. *DNA-dependent ATPase activities after introduction of the rep$^+$-containing plasmid pLC44-7 into the E. coli HF4704 rep$^-$ strain. See legend to Fig. 2 for definition of symbols. Extracts were prepared from 6 × 10^{11} cells.*

the *rep* activity was lost or altered in the presence of chloramphenicol suggests that either a very minimal level of protein synthesis is required or that the phage injects a protein inhibitor together with DNA. That chloramphenicol was inhibiting phage protein synthesis is indicated by the absence of the phage-induced ATPase in the peak 5 region. The possibility of a protein inhibitor being injected with the DNA is mentioned because the T4 *alt* protein, which ADP ribosylates RNA polymerase, functions in this way (13, 26).

T4 phage-induced DNA-dependent ATPases. In an earlier section, we noted the appearance of a T4 phage-induced DNA-dependent ATPase in peak 5 (Fig. 1). This ATPase is the gene 44/62 protein described by Piperno et al. (24), as this activity is missing in *am* mutants in genes 44 and 62 (data not shown). The fact that this enzyme was easily separable from the other DNA-dependent ATPases by phosphocellulose chromatography provides an alternative route for the purification of the 44/62 protein as an ATPase without recourse to the more complex complementation assay (22, 23). The *dda* DNA-dependent ATPase eluted in the region of peak 2 and could be separated from the host enzyme with a shallow KCl gradient (data not shown).

DISCUSSION

***rep* and the *recBC* functions.** Previous studies have indicated that the *recBC* nuclease, a DNA-dependent ATPase, is inhibited by a lower-molecular-weight, T4 phage-induced protein (3). The studies here indicated that another host DNA-dependent ATPase, the *rep* enzyme, is also inhibited by T4, but the mechanism of inhibition is not known. Interestingly, both enzymes are DNA-dependent ATPases which have DNA unwinding activity (16, 27, 33). The *recB*, and *recC*, and *rep* mutants are all UV sensitive (7, 35). Whereas both *recB* and *recC* mutants are clearly defective in recombination (5, 35), the *rep* mutants have been reported to have either a wild-type level of recombination or levels of recombination ranging from ⅓ to 1/35 those of the wild-type strains (4, 7, 8). The possibility that the *recBC* and *rep* proteins might be functionally related was considered because of an observation by Lieberman and Oishi (19) that treatment of *recBC* nuclease with high salt dissociated a protein which was not coded by the *recB* or *recC* genes and which was necessary for activity. This protein had a molecular weight of approximately 60,000, as determined by glycerol gradient sedimentation. This value is not too different from the molecular weight of 65,000 to 70,000 assigned to the *rep* protein by sodium dodecyl sulfate-polyacrylamide gel analysis (29, 33). If the *rep* protein were a component of *recBC* nuclease, it would be a convenient means of accounting for the inhibition of the two enzymes by T4 phage infection and other common properties. However, the *rep* mutant, strain HF4704 *rep*$^-$, and wild-type strain HF4704 have comparable *recBC* nuclease activities, suggesting that the *rep* activity is not essential for *recBC* nuclease activity (Thalia Assuras, personal communication).

Why inhibit *rep*? What is the physiological significance of the inhibition of the *rep* function by T4 phage? A view we favor is that by inhibiting the *rep* function, the phage might be able to turn off host DNA replication. At present this possibility is difficult to assess because the requirement and specificity of *rep* in host DNA replication are unclear. *rep* mutants studied so far are lethal for φX174 but not for the host (7). However, *rep* mutants have abnormalities in their nucleoid structures and have slower movement of replication forks, clearly indicating the involvement of *rep* in host DNA replication (17, 18). It is not known whether *rep* mutants are lethal for φX174 but not for *E. coli* because of a greater requirement for the *rep* function in φX174 replication and whether critical mutants have yet to be isolated to test *rep* for lethality

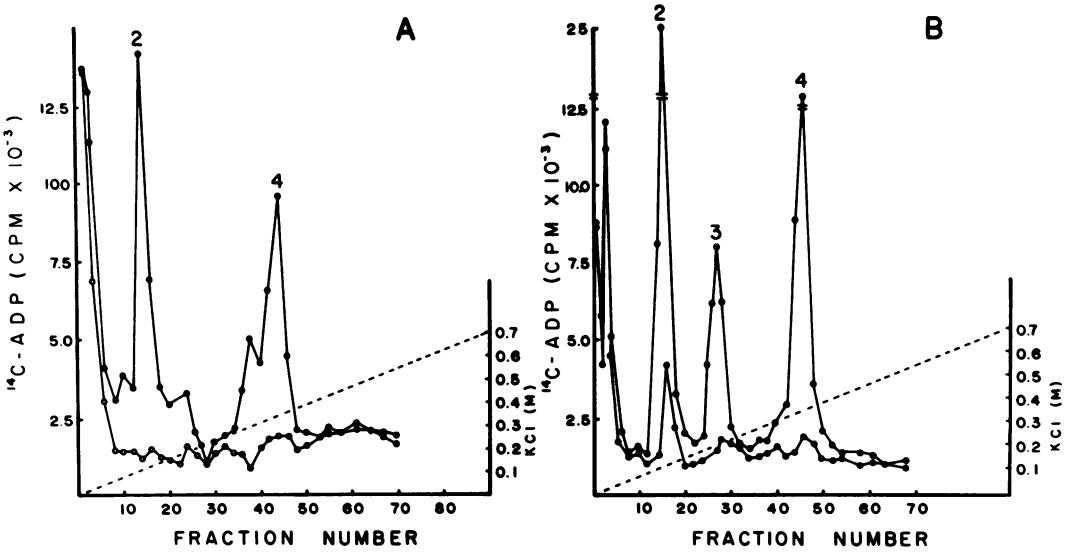

FIG. 4. *Effect of chloramphenicol on DNA-dependent ATPase activities. (A) Enzyme activities from extracts of E. coli strain B infected with T4amE1140 (gene 62) in the presence of chloramphenicol. (B) All treatments were identical to those in A except for the omission of the phage. See legend to Fig. 2 for definition of symbols. Extracts were prepared from 3 × 10^{11} cells.*

with *E. coli*. Alternatively, the host might have a backup system for the *rep* function. The inhibition of *rep* by T4 phage suggests that T4 phage codes for its own *rep*-like function. If so, it would argue for specificity in the *rep* function.

Although the physiological consequences of the inhibition of the *rep* function are unknown, the observations here support the view that phage functions directly react with and possibly control the DNA replication machinery of the host. The inhibition of *rep* may be another example of a general mechanism by which a phage controls host macromolecular synthesis by altering or inhibiting key components in the synthetic pathway.

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